Catalog #90105

V.3

Intact Proinsulin ELISA Kit Instructions

For the quantitative determination of intact proinsulin in human serum and plasma

Catalog #90105
96 Assays

For research use only. Not for use in diagnostic procedures.

Crystal Chem, Inc.
955 Busse Road
Elk Grove Village, IL 60007, USA
Tel: (630) 889-9003 Fax: (630) 889-9021
E-mail: sales@crystalchem.com
Order online: www.crystalchem.com

Catalog #90105

TABLE OF CONTENTS

A. Intended Use

The Intact Proinsulin ELISA kit is for the quantitative determination of intact proinsulin in human serum and plasma. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Proinsulin is synthesized by the β-cell of the pancreas as a precursor molecule for insulin. Normally, proinsulin is rapidly degraded in the bloodstream. An increase in the insulin demand, as provided by insulin resistance in later stages of type 2 diabetes mellitus or in patients with insulinoma, can result in increased expression of proinsulin. Monitoring the intact proinsulin values using a highly specific assay may serve as a predicative indicator for monitoring such situations and could be used to serve as the basis for the selection of an insulin resistance therapy.

C. Principle of the Assay

The Intact Proinsulin ELISA kit is an ELISA sandwich assay for intact proinsulin and utilizes a specific antibody immobilized onto the microplate wells and an antibody labeled with HRP. In the first step, intact proinsulin in the sample binds to the antibody coated microtiter plate. After washing, an HRP labeled antibody is added and forms a complex with the intact proinsulin bound to the well. After another wash step, TMB substrate is added and the concentration of intact proinsulin is measured based on the resulting color intensity.

D. Kit Storage

1. Upon receipt of the Intact Proinsulin ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 - Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
STD1-5	Standards (Lyophilized)	5 x 1 vial
CON1-2	Controls (Lyophilized)	2 x 1 vial
HRP	HRP Conjugate	12 mL
BUF	Sample Buffer	12 mL
WASH	Wash Buffer (30X Concentrate)	50 mL
SUB	Substrate (TMB) Solution	12 mL
STOP	Stop Solution	12 mL

E.2. Materials required but not provided

Micropipettes and disposable tips
Deionized water
Microplate sealers
Polypropylene microtubes
Vortex mixer
Microplate reader (capable of reading A_{450} and $A_{620/650}$ values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the small sample volumes required (50 µL), pipetting should be done as carefully as possible. A high quality 100 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each standard and sample should be assayed in duplicate. The same sequence of pipetting and other operations should be maintained in all procedures.
4. Do not mix reagents that have different lot numbers.
5. Avoid microbial contamination to minimize false results.

H. Sample Collection

It is recommended that plasma samples be used. For plasma samples, whole blood samples should be collected with EDTA or heparin anticoagulant and immediately centrifuged after collection. For serum samples, whole blood should be allowed to clot over 30 minutes and then the clot removed via centrifugation prior to use. Hemolytic samples should be avoided. Serum samples should be frozen if they are not going to be immediately assayed, but plasma can be stored at 2-8°C for up to 24 hours. For long-term storage (>24 hours) of plasma, samples should be stored at -20°C. Avoid repeated freeze-thaw cycles of samples. Thawed samples should be inverted several times prior to testing.

I. Assay Procedure

I.1. Preparation of reagents

Prior to preparing any materials, all reagents should be brought to room temperature before use.

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture. Unused wells are stable for 2 months if stored at 2-8°C.
2. Standards 1-5
   Standards are provided in lyophilized form with concentrations ranging from 0.0 pmol/L to 110 pmol/L. Dilute each standard with 1 mL of deionized water. After reconstitution, it is recommended that standards be allowed to sit for 5 mins at room temperature and then mixed thoroughly, but gently, with a Vortex mixer to dissolve all solids. Reconstituted standards are stable for four weeks at 2-8°C.
3. Controls 1-2
   Two controls are provided (high and low concentration) in lyophilized form with target values and ranges included on their labels. Dilute each control with 1mL of deionized water. After reconstitution, it is recommended that controls be allowed to sit for 5 mins at room temperature and then mixed thoroughly, but gently, with a Vortex mixer to dissolve all solids. Reconstituted controls are stable for four weeks at 2-8°C. The controls can be further diluted to establish a relevant working range unique to the end-users application.
4. HRP Conjugate
   Provided as ready to use.
5. Sample Buffer
   Provided as ready to use. Includes 0.05% sodium azide and other preservatives.
6. Wash Buffer (30X Concentrated)
   Prepare a working concentration of buffer by diluting 1 part of Wash Buffer with 29 parts of distilled or deionized water. For example, 50 mL of wash buffer must be diluted with 1450 mL of deionized or distilled water. Wash buffer is stable for 4 weeks at 2-8°C after dilution, so dilute only as needed.
7. Substrate Solution
   Provided as ready to use.
8. Stop Solution
   Provided as ready to use.

I.2. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. Add 50 µL of Sample Buffer and 50 µL of sample, standard, or control to each well, and mix by repeated pipetting.
2. Seal the plate, and incubate for 1 hour at room temperature on a plate shaker at moderate speed (600 rpm). If a plate shaker is

unavailable or samples have very high rheumatoid factor (RF), incubate without shaking for 2 hours.

3. Remove the plate seal, and aspirate well contents. Wash three times using 300 µL of working strength Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
4. Add 100 µL of HRP Conjugate in each well, and then gently agitate the plate to mix.
5. Seal the plate, and incubate the plate for 1 hour at room temperature on a plate shaker (600 rpm). If a plate shaker is unavailable, incubate at room temperature, without shaking, for 1 hour.
6. Remove the plate seal, and aspirate well contents. Wash three times using 300 µL of working strength Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 µL of Substrate Solution in each well, and then gently agitate the plate to mix.
8. Seal the plate, and incubate the plate for 15 minutes at room temperature in the dark.
9. Remove the seal, and stop the reaction by adding 100 µL of Stop Solution to each well.
10. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values with the optical density (OD) normalized by subtracting $A_{620/650}$ values).

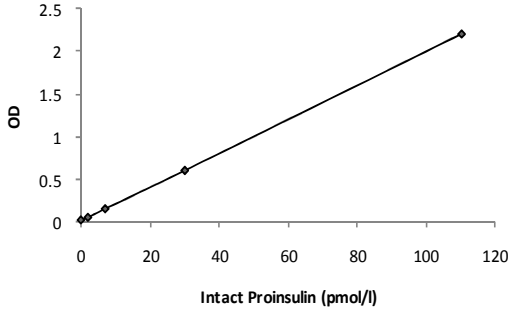
I.3. Determining the Intact Proinsulin (pmol/L)

1. Using computer software, construct the intact proinsulin calibration curve by plotting the mean optical density for each standard on the Y axis versus the corresponding intact proinsulin concentration (pmol/L) on the X axis, Figure 1. Using a cubic spline or 4-parameter curve fit, sample concentrations can be read directly from the calibration curve. Other types of data processing fit functions may give slightly different results.

   Note: *A calibration curve should be plotted every time the assay is performed.*

Figure 1 – Example Calibration Curve

For illustration only. Do not use for your samples.



2. Proinsulin concentrations in the samples are interpolated using the calibration curve and mean optical density values for each sample. The proinsulin concentration is expressed in pmol/L.

J. Performance characteristics

J.1. Assay range

The Intact Proinsulin ELISA Kit has an assay range from 0.3 – 100 pmol/L.

J.2. Precision

The assay has a within-run and total precision of CV <10%, and the table below indicates the percent cross reactivity observed in the assay.

TABLE 2 – Cross Reactivity

Analyte	Cross Reactivity (%)
Intact Proinsulin	100%
Insulin	0%
C-Peptide	0%
32-33 split proinsulin	5.6%
Des 31-32 split proinsulin	1.4%

Warranty

Crystal Chem Inc. makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Buyer assumes all risk and liability resulting from the use of this product.

THERE IS NO WARRANTY OF MERCHANTABILITY OF THE PRODUCTS, OR THAT SUCH PRODUCTS ARE FIT FOR ANY PARTICULAR PURPOSE. CRYSTAL CHEM INC'S LIABILITY SHALL NOT EXCEED THE RETURN OF THE PURCHASE PRICE, AND UNDER NO CIRCUMSTANCES SHALL CRYSTAL CHEM INC. BE LIABLE FOR SPECIAL OR CONSEQUENTIAL DAMAGES, OR EXPENSES ARISING DIRECTLY OR INDIRECTLY FROM THE USE OF THIS PRODUCT.